Effect of triacontanol on the pharmacokinetics of docetaxel in rats associated with induction of cytochrome P450 3A1/2

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Abstract

1. Triacontanol was confirmed to have a potential anti-cancer effect, the aim was to assess whether the co-administration of triacontanol alters the exposure of docetaxel via inducing hepatic CYP3A1/2 activity. The concentration of docetaxel in rats pretreated with triacontanol for seven successive days was determined, and the expression levels of CYP3A protein and mRNA were analyzed by the western blot and real time polymerase chain reaction (RT-PCR) technique, respectively.

2. The concentrations of docetaxel in rats treated with triacontanol were decreased, with 61.5%, 61.9% decrease in AUC0–24h and 65.7%, 54.9% reduction in Cmax (120 and 180 mg kg⁻¹, respectively) compared with the control. Hepatic clearance of docetaxel was enhanced in vitro and in vivo at dosage of 120 and 180 mg kg⁻¹, and CYP3A activity was upregulated by measuring the formation rate of 1-hydroxymidazolam. Triacontanol preferentially induced protein expression level of CYP3A2 in a dose-dependent manner and of CYP3A1 at dosage of 120 and 180 mg kg⁻¹. The mRNA expression of CYP3A1 was moderately different with the western blot results, but the trends appeared similar. CYP3A2 mRNA level was not markedly affected by triacontanol.

3. The significant triacontanol–docetaxel interaction was largely due to the induction of CYP3A1/2, which brought useful information in the clinical therapy when the combination is administered in human.

Introduction

Triacontanol (Figure 1), a member of very long chain fatty alcohols, is a component of policosanol isolated from rice bran, sugar cane wax, wheat germ and beeswax (Dullens et al., 2008). Previous studies showed that policosanol could lower the total cholesterol level (Aneiros et al., 1995; Gouni-Berthold & Berthold, 2002), inhibit platelet aggregation (Arruzazabala et al., 1992, 2002) and lipid peroxidation (Menendez et al., 1997) in both experimental models and humans. As an ingredient of policosanol, triacontanol shows a remarkable effect in inhibiting cholesterol synthesis in cultured rat hepatoma cells (Singh et al., 2006). Recently, Fan et al. demonstrate that triacontanol has prominent anticancer effects, which follows obvious dose–effect relationships, especially in conditions of liver cancer, intestinal cancer and lung cancer. Meanwhile, adverse effects on the important immune organs of the tumor-bearing mice were not observed after an administration at a dose of 150 mg/kg/d (Fan et al., 2011). For limited resources, the costs of the conventional treatments to treat cancer, including surgeries and radiotherapy remain prohibitively high to the majority of people in the developing countries (Fei et al., 2010). Hence, triacontanol is a promising compound in the treatment of cancer, for its relatively abundance resources in cereal grains, bran and germ, as well as in leaves, seeds and nuts (Hargrove et al., 2004), easy affordability and less or no toxic effects (Zhang et al., 2008).

Docetaxel, belonging to the same class as paclitaxel, interferes with cell division via disrupting the micro-tubular network. It promotes the stability of microtubules, resulting in the inhibition of mitosis in cells (Yamamoto et al., 2000). This compound has been successfully used in breast, ovarian and lung cancer therapy (Huizing et al., 1995; Klener, 1995; Rowinsky et al., 1992). However, the unpredictable interindividual variation in efficacy and toxicity of docetaxel is a major limiting factor in docetaxel clinical application, which can be attributed to the variability of individual metabolism and elimination of this compound (Bruno et al., 1996; Hudachek & Gustafson, 2013). The metabolism of docetaxel...
is predominantly mediated by the cytochrome P450 (CYP450) family member CYP3A (Marre et al., 1996). Drugs and dietary supplements with influences on CYP3A activity may affect the pharmacokinetics of docetaxel, thus resulting in unsatisfactory therapeutic efficacy. CYP3A plays a major role in the metabolism of more than 50% of the marketed drugs (Guengerich, 1999). Drug–drug interactions (DDIs), mediated by induction of CYP3A, may cause undesirable therapeutic efficacy of co-administrated drugs. Therefore, it is of great importance to estimate the DDIs mediated by the enzyme induction potential for a new compound during drug development (Guo et al., 2013; Tettey et al., 2001).Scarce data involving the potential effect of triacontanol as an anti-cancer drug on metabolic enzymes, especially on the expression levels of CYP3A protein and mRNA, have been reported. Hence, it is necessary to clarify whether the co-administration of triacontanol with docetaxel could affect the pharmacokinetics of the latter.

The current study aims to assess potential influence of triacontanol on the pharmacokinetics of docetaxel, and to study the mechanisms of the interaction. In the present study, rats were gavaged with triacontanol (60, 120 and 180 mg kg$^{-1}$) for seven consecutive days. On the seventh day, docetaxel was injected into the above-mentioned rats at a dose of 5 mg/kg, and then plasma concentration profiles of docetaxel were then determined. The possible involvement of CYP3A1/2 in triacontanol and docetaxel interaction was analyzed by determining the CYP3A activity, protein and mRNA expression levels.

Materials and methods

Chemicals and reagents

Triacontanol was supplied by Kunming Longjin Pharmaceutical Co., Ltd (Kunming, China). Docetaxel was purchased from Shanghai Sunve Pharmaceutical Co., Ltd. (Shanghai, China). Paclitaxel (the internal standard) was obtained from Sigma Chemical Co. (St. Louis, MO). Anti-glyceraldehyde-3-phosphate dehydrogenase GAPDH (6C5) was obtained from CWBiotech (Beijing, China). Rabbit polyclonal antibodies for CYP450 3A1 (BML-CR3310) and 3A2 (BML-CR3320) were purchased from Enzo Life Sciences (New York). Goat anti-rabbit IgG antibody conjugated with horseradish peroxidase (HRP) was purchased from Bioworld technology Co. Ltd. (Minnesota). TRizol Reagent was purchased from Invitrogen (Carlsbad, CA). Transcriptor first strand cDNA synthesis Kit and FS Universal SYBR Green Master were purchased from Roche (Mannheim, Germany). Bicinchoninic acid (BCA) assay commercial kits were purchased from CWBiotech (Beijing, China). HPLC-grade methanol and acetonitrile were purchased from Tedia (Fairfield, OH).

Animals

Sprague-Dawley rats (male and female, 200 ± 10 g) were purchased from Shanghai SIPPR/BK Experimental Animal Company Ltd. All experimental procedures were conducted following an approval from the Animal Ethics Committee of China Pharmaceutical University. The animals were kept in a temperature controlled room (20 ± 2 °C) with free access to food and water.

Pharmacokinetic studies of docetaxel

Thirty rats were randomly divided into five groups, including vehicle controls, positive controls and three experimental groups (six rats in each group). Males and females in each group were the same in number. The normal control group was administered 0.5% sodium carboxymethylcellulose (CMC-Na) via the oral route, while the positive control group received daily intraperitoneal injection of phenobarbital at a dose of 75 mg kg$^{-1}$. Three experimental groups were orally administered triacontanol suspension at a dose of 60, 120 and 180 mg/kg, respectively. The treatments of each group were given for seven consecutive days. On the seventh day, all rats were given a single intravenous (i.v.) injection of docetaxel at the dosage of 5 mg kg$^{-1}$ an hour after the last administration of triacontanol. About 0.1 mL blood samples were collected from jugular vein into tubes pre-treated with 10 µL heparin sodium at 0.0833, 0.166, 0.322, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 and 24 h, centrifuged (5 min, ×8000g) and stored at −80°C until analysis.

Preparation of liver tissue and microsomes

After the last blood sampling, rats were sacrificed and the liver was immediately collected and drenched with ice-cold physiological buffered saline. One part of the liver was used for the separation of mRNA, another for preparation of liver microsomes which were used for western blot and enzyme activities assay. Microsomes were prepared using the method of differential centrifugation (Gibson & Skett, 1994). All samples were frozen in liquid nitrogen and stored at −80°C until use. BCA commercial kits were used for determining protein concentrations.

Metabolism of docetaxel in rat liver microsomes

To further confirm the enhanced clearance of docetaxel in triacontanol pre-treated rats was due to the increase in hepatic enzyme activity, we investigated the biotransformation of docetaxel in rat liver microsomes (Li et al., 2011; Royer et al., 1996). Docetaxel (2 µM) was incubated in the reaction system which contained 0.5 mg/mL rat liver microsomes, 0.1 M phosphate buffer (pH 7.4), 5 mM MgCl$_2$, 1 mM β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt (NADPH) in a final volume of 200 µL. After the microsomes and docetaxel pre-incubating at 37°C for 5 min, 1 mM NADPH was added to initiate the reaction at 37°C for 30 min. About 0.4 mL ice-cold ethyl acetate containing with 2 ng/mL paclitaxel (internal standards) was added to terminate the reaction. The mixture was centrifuged at 12 000g for 5 min, and then 10 µL supernatant was analyzed by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Measurement of CYP3A activity in rat liver microsomes

The ability of triacontanol of different doses to induce the CYP3A was investigated in rat liver microsomes, midazolam was used as a probe substrate which was incubated in the reaction system mentioned above, and the formation rates of 1-hydroxymidazolam were determined for CYP3A activity. A 5 min pre-incubation was conducted before the reaction was started by the addition of 1 mM NADPH, after 5 min at 37 °C, 0.4 mL ice-cold ethyl acetate with 2 ng/mL diazepam (internal standards) was added to terminate the reaction. The mixture was centrifuged at 12,000 g for 5 min, and then 10 μL supernatant was analyzed using LC-MS-MS.

Determination of CYP3A1/2 by western blot

Liver microsomes (100 μg per lane) were isolated by 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to polyvinylidene fluoride membranes, which were blocked at room temperature for 2 h in 5% nonfat milk (dissolved in TBST: tris-buffered saline, with 0.1% Tween 20; pH 7.4). The membranes were then incubated with rabbit polyclonal anti-CYP3A1 (1:2,000), or rabbit polyclonal anti-CYP3A2 (1:2,000), or rabbit monoclonal anti-GAPDH (1:1,000) for 1 h at room temperature and overnight at 4 °C. After being washed three times with TBST (each time for 10 min), the membranes were then probed with secondary antibody conjugated HRP (1:5,000) for 1 h at room temperature. The blots were washed three times with TBST, every 10 min. The immune-reactive bands were detected and visualized on radiographic film.

Measurement of 3A1/2 mRNA

Total RNA of liver tissue was isolated by TRIzol reagent according to the manufacturer’s protocol. The RNA concentration was determined, and the 260/280 nm absorbance ratio was used to evaluate the quality of extracted RNA samples (range from 1.8 to 2.0) (Pan et al., 2002). Complementary DNA was synthesized using Transcriptor first strand cDNA synthesis Kit following the supplier’s instructions, and stored at −20 °C until analysis. Real-time-PCR (RT-PCR) was performed for the determination of CYP3A1/2 mRNA expression levels using the SYBR Green detection system. RT-PCR was conducted in a reaction mixture of 10 μL. Reaction systems for determining the amounts of CYP3A1/2 and GAPDH mRNA were composed of Real Master Mix/SYBR (5 μL), 0.5 μL of cDNA, 2 μL mixtures of the forward and reverse primers (0.3 mM) and 2.5 μL double distilled water. The reverse transcription reaction was incubated at 95 °C for 10 min, then the following action was performed for 40 cycles: denaturation at 95 °C for 15 s, 30 s annealing at 58 °C (3A2), 60 °C (3A1,GAPDH) and extension at 60 °C for 60 s. The relative mRNA levels were quantified by the 2^{-ΔΔCt} method (Livak & Schmittgen, 2001). Specific primer pairs used in this study are as follows (Li et al., 2012):

- For CYP3A1:
  Forward: 5'-GGAAATTCGATGGAGTGTC-3'
  Reverse: 5'-AGGTTCGCTTTTCTTTGCCC-3'

- For CYP3A2:

Analytical methods

For rat subjects, about 50 μL harvested plasma was transferred to the triple volume of acetonitrile containing 2 ng/mL paclitaxel (IS) immediately, Vortexed for 3 min and centrifuged at 12,000 g for 5 min. An aliquot of 10 μL of the supernatant was injected into the LC-MS/MS system for analysis.

Concentrations of 1-hydroxymidazolam and docetaxel in the biological samples were determined by LC-MS/MS using the established methods (Li et al., 2011; Wang et al., 2013). LC-MS/MS studies were performed using a Thermo Scientific TSQ Quantum MS/MS system equipped with electrospray ionization (ESI) source in positive-ion mode. Quantification was accomplished in selective reaction monitoring. For the determination of docetaxel, the mobile phase is composed of phase A (0.1% formic acid and 0.3 mM sodium acetate) and phase B (methanol). For 1-hydroxymidazolam, the mobile phase consisted of purified water (phase A) and acetonitrile (phase B). The flow rate was 0.3 mL/min; gradient mode of elution was used to achieve chromatographic separation. Chromatographic conditions and mass conditions for the substrate were summarized in Table 1.

Statistical analysis

A non-compartmental model was used to analyze the pharmacokinetic profiles of docetaxel, the pharmacokinetic parameters were calculated by WinNonlin Professional Version 5.0.1 (Pharsight Corp, Mountain View, CA). Statistical analyses were conducted using the SPSS17.0 (SPSS Inc., Chicago, IL). Individual variance (one-way analysis of variance) was evaluated using Dunn’s test. All data were shown as mean ± SD p < 0.05 were considered significant difference.

Results

At the end of the study, the body weight of rat subjects increased 2.1%, 3.3%, 5.9% and 13.3% in low-, middle- and high-dose group and positive control group, respectively, compared with vehicle controls. However, there are no significant differences within and between groups. No adverse effects on rats administrated with triacontanol for seven consecutive days were observed. The result was in accordance with previous report in which the LD50 of triacontanol were over 5000 mg/kg by oral in rats (Zhang et al., 2008).

Validation of analytical method

The calibration curve of docetaxel was linear over the range of 1–1000 ng/mL with the lower limit of quantification of 1 ng/mL (R^2 > 0.999). The relative standard deviation of inter-day and intra-day precision was both within 15% of the 3 QC levels (2, 50 and 800 ng/mL), and the derivation of accuracy was all within 100 ± 10% of the actual values. There was no significant matrix effect observed. The LC-MS/MS
method described above has been proved to be sensitive, and selective for determination of docetaxel in rat plasma samples.

**Effect of triacontanol on the pharmacokinetics of docetaxel**

The plasma concentration profiles of docetaxel were shown in Figure 2, and the mean pharmacokinetic parameters estimated for docetaxel were summarized in Table 2. Compared with the control group, area under concentration–time curve (AUC\(_{0-24h}\)) dropped to 61.5%, 61.9%, the maximum plasma concentration (C\(_{\text{max}}\)) reduced to 65.7%, 54.9% and the clearance increased up to 1.68- and 1.69-fold for the middle-dose triacontanol group (120 mg kg\(^{-1}\), Figure 2B) and high-dose groups (180 mg kg\(^{-1}\), Figure 2C), respectively. At the same time, phenobarbital-pretreated rats displayed a 2.82-fold increase in clearance of docetaxel, with the C\(_{\text{max}}\) and AUC\(_{0-24h}\) falling to 41.7%, 37.8% compared with control group, respectively (Table 2 and Figure 2D).

### Table 1. HPLC and MS/MS condition for docetaxel and 1-hydroxymidazolam.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Column</th>
<th>HPLC Mobile phase (B%)</th>
<th>Q1/Q3 (m/z)</th>
<th>MS/MS TEP (±)</th>
<th>SV (v)</th>
<th>SGP (Arb)</th>
<th>AGP (Arb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docetaxel</td>
<td>BDS HYPERSIL C18</td>
<td>0–1.0 min, 10%</td>
<td>830.3→549.0</td>
<td>350</td>
<td>3500</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>Paclitaxel (IS)</td>
<td>Thermo Scientific</td>
<td>2.0–4.5 min, 90%</td>
<td>876.0→307.8</td>
<td>350</td>
<td>3500</td>
<td>40Arb</td>
<td>15Arb</td>
</tr>
<tr>
<td>1-Hydroxymidazol</td>
<td>Xtimate C18</td>
<td>0.01–1.00 min, 30%</td>
<td>342.2→168.1</td>
<td>350</td>
<td>3500</td>
<td>40Arb</td>
<td>15Arb</td>
</tr>
<tr>
<td>Diazepam (IS)</td>
<td>Welch Materials</td>
<td>1.50–3.50 min, 90%</td>
<td>285.1→193.2</td>
<td>350</td>
<td>3500</td>
<td>40Arb</td>
<td>15Arb</td>
</tr>
</tbody>
</table>

IS, Internal standard; SV, spray voltage; TEM, temperature; SGP, sheath gas pressure; AGP, auxiliary gas pressure; HPLC, high-performance liquid chromatography.
parameters in low-dose group had no significant changes compared with the vehicle controls (Table 2 and Figure 2A). The value of $t_{1/2}$ in both triacontanol-pretreated groups and phenobarbital group was similar with that in the vehicle controls (Table 2).

Enhanced hepatic clearance of docetaxel in rat liver microsomes

Since the hydroxydocetaxel is not commercially available, the biotransformation of docetaxel in rat liver microsomes was evaluated by the determination of remaining docetaxel in the incubation system after reaction was terminated. Figure 3 showed that the metabolism of docetaxel in vitro was enhanced after treatment with triacontanol (120 and 180 mg kg$^{-1}$). Moreover, the most obvious induction was observed at the middle dose group (120 mg kg$^{-1}$). It was confirmed that hepatic clearance of docetaxel in rat liver microsomes was up-regulated, which was in accordance with the decreased exposure of docetaxel in rats pre-treated with triacontanol. Thus, we might conclude that the enhanced clearance of docetaxel in rats administrated with triacontanol was due to the increase in hepatic enzyme activity.

Induced hepatic CYP3A activity

The formation rates of 1-hydroxymidazolam could reveal the changes in CYP3A activity. Figure 4 indicated that the enhancements of enzyme activities by triacontanol were dose dependent. The biotransformation rates of midazolam between three triacontanol groups and phenobarbital group were faster than that in the vehicle controls. Hence, we concluded that hepatic CYP3A activity was up-regulated by triacontanol.

Effect of triacontanol on the CYP3A1/2 protein expression

The western blot analysis was carried out to evaluate the effect of triacontanol on the protein expression levels of CYP3A1/2. Specific bands were detected at around 50 kDa for CYP3A1 and CYP3A2 (Figure 5A). As shown in Figure 5(A) (top), the expression level of CYP3A1 in the

### Table 2. Pharmacokinetic parameters of docetaxel in triacontanol pretreated rats (mean ± SD, n = 6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>Control</th>
<th>Low-dose (60 mg kg$^{-1}$)</th>
<th>Middle-dose (120 mg kg$^{-1}$)</th>
<th>High-dose (180 mg kg$^{-1}$)</th>
<th>Phenobarbital (75 mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C$_{\text{max}}$ (µg L$^{-1}$)</td>
<td>1290.0 ± 226.4</td>
<td>1178.9 ± 249.9</td>
<td>847.3 ± 102.8**</td>
<td>707.9 ± 273.3***</td>
<td>538.0 ± 134.7***</td>
</tr>
<tr>
<td></td>
<td>MRT$_{0-24}$ (h)</td>
<td>4.2 ± 0.2</td>
<td>4.1 ± 0.2</td>
<td>3.8 ± 0.5</td>
<td>3.8 ± 0.2</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>CLz (L h$^{-1}$ kg$^{-1}$)</td>
<td>4.628 ± 0.361</td>
<td>4.891 ± 0.694</td>
<td>7.802 ± 0.910**</td>
<td>7.809 ± 0.749**</td>
<td>13.029 ± 3.359***</td>
</tr>
<tr>
<td></td>
<td>AUC$_{0-24}$ (µg h L$^{-1}$)</td>
<td>1015.0 ± 33.6</td>
<td>977.1 ± 151.6</td>
<td>629.1 ± 65.5**</td>
<td>624.9 ± 51.8**</td>
<td>383.2 ± 87.3***</td>
</tr>
<tr>
<td></td>
<td>AUC$_{0-\infty}$ (µg h L$^{-1}$)</td>
<td>1085.9 ± 83.5</td>
<td>1038.8 ± 141.0</td>
<td>648.6 ± 80.2**</td>
<td>645.5 ± 64.8**</td>
<td>404.3 ± 99.0***</td>
</tr>
</tbody>
</table>

***Significantly different from control, $p < 0.001$.

**Significantly different from control, $p < 0.01$.

Figure 3. Metabolism of docetaxel in rat liver microsomes pre-treated with triacontanol for seven days. Rats were pretreated for seven days with triacontanol (60, 120 and 180 mg kg$^{-1}$) or phenobarbital (75 mg kg$^{-1}$) and the control group. Phenobarbital group was used as the positive group. The metabolism of docetaxel is shown as percentage of parent compound remaining. Data are mean ± SD. n = 3. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ versus control.

Figure 4. CYP3A activity in rat liver microsomes pre-treated with triacontanol for seven days. Rats were pretreated for seven days with triacontanol (60, 120 and 180 mg kg$^{-1}$) or phenobarbital (75 mg kg$^{-1}$) and the control group. Phenobarbital group was used as the positive group. The activity of CYP3A was evaluated by the formation rate of 1-hydroxymidazolam. Data are mean ± SD. n = 3. **$p < 0.01$; ***$p < 0.001$ versus control.
Effect of triacontanol on CYP3A1/2 mRNA expression

The mRNA expression levels of CYP3A1/2 were measured by RT-PCR (Figure 6). Compared with the vehicle controls, treatment with triacontanol (120 mg kg\(^{-1}\)) remarkably enhanced the mRNA expression of CYP3A1 (4.8-fold, \(p<0.01\)), while mRNA level of CYP3A1 appeared to decrease at the dose of 180 mg kg\(^{-1}\) (0.086-fold, \(p<0.01\)). These results were in accordance with our observations in the western blotting experiment where the protein expression level of CYP3A1 in middle-dose of the triacontanol group was higher than that in high-dose group. In addition, CYP3A2 mRNA level was not significantly affected by triacontanol in either male or female rats, except for the increase in CYP3A2 mRNA in high-dose group (3.6-fold, \(p=0.082\)).

Middle-dose (120 mg kg\(^{-1}\)) group was significantly higher than that of in high-dose (180 mg kg\(^{-1}\)) group. The protein expression of CYP3A2 was significantly increased in a concentration-dependent manner in all three triacontanol groups (Figure 5A (bottom)). The total protein expression levels of 3A were almost the same in high- and middle-dose of triacontanol groups, which could be interpreted as similar pharmacokinetics profiles of docetaxel in these two groups. The levels of 3A1/2 were also increased in the low-dose (60 mg kg\(^{-1}\)) group, but no significant effect was observed on the pharmacokinetics of docetaxel (Figure 2A) and on the metabolism of docetaxel in vitro (Figure 3).

Discussion

The combination therapy to optimize treatment effect on cancer, such as capecitabine, cisplatin and epirubicin, has been approved by the US Food and Drug Administration. Even thought combination therapy contributes to improved efficacy, it causes some potential toxicity (Miles et al., 2002; Tkaczuk 2009). Adverse effects are more likely to occur when the co-administered drugs are inducers or inhibitors of CYP450, especially CYP3A, which can be induced or inhibited by many xenobiotics, thus becoming a preferential target for DDIs and leading to therapeutic failure. Consequently, it is necessary to evaluate the potential of enzyme induction in the stage of new drug development (Güengerich, 1999; Guo et al., 2013; Tettey et al., 2001). In the present study, the interaction between triacontanol and docetaxel was performed in rats. Research findings suggested that administration of triacontanol for seven days obviously reduce the exposure of docetaxel in the circulation system. Studies in vitro implicated that interaction between triacontanol and docetaxel was in connection with the up-regulation of hepatic CYP3A1/2. This is the first report to illustrate the effects of triacontanol, as an anti-cancer drug, on the induction of CYP3A1/2 in rats.

In this study, the \(t_{1/2}\) value of docetaxel, changing from 3.6 to 4.2h, was in accordance with values published before (Li et al., 2011). Considering the results in vivo, we assumed that the influence of triacontanol on the pharmacokinetics of docetaxel in rats may be mediated by inducing hepatic CYP3A. This hypothesis was supported by the facts that pretreatment with triacontanol led to the significant reduction in both \(C_{\text{max}}\) and AUC\(_{0\rightarrow24\text{h}}\) and the increase in the clearance of docetaxel. Furthermore, the observation obtained in the phenobarbital (an inducer of CYP450s) group was similar to that in triacontanol pre-treated groups. In addition,
Marre et al. (1996) demonstrated that the metabolism of docetaxel is predominantly mediated by the CYP3A. Combining these results together, we inferred that CYP3A may be induced by triacontanol, thus enhancing hepatic drug metabolism and reducing the exposure of docetaxel in systemic circulation.

1-Hydroxymidazolam is predominantly catalyzed by CYP450 3A1 and 3A2 (Kobayashi et al., 2002), thus, the influence of triacontanol on CYP3A activity was assessed by the formation rate of 1-hydroxymidazolam in vitro. The metabolism of midazolam in any triacontanol groups increased in a dose-dependent manner, which suggested that CYP3A activity was up-regulated by triacontanol. Besides, the clearance of docetaxel was enhanced in rat liver microsomes in triacontanol pretreated groups, which was consistent with the results in vivo. Considering the fact that the metabolism of docetaxel is mainly mediated by CYP3A (Marre et al., 1996; Royer et al., 1996), we may further confirmed the hypothesis that the increase in the clearance of docetaxel was due to the induction of CYP3A. However, docetaxel was converted into four metabolites in microsomes and the biotransformation of docetaxel could be inhibited by typical CYP3A substrates and/or inhibitors such as erythromycin, ketoconazole, nifedipine, midazolam and troleandomycin (Marre et al., 1996; Royer et al., 1996), which indicated the existence of CYP3A1/2 multiple binding sites (Khan et al., 2002) or the genetic polymorphism. These findings made it easy for us to understand the different biotransformation rates of midazolam and docetaxel in rat microsomes pretreated with triacontanol (180 mg/kg).

To further illustrate the underlying mechanisms of the interaction between triacontanol and docetaxel, we determined protein expression levels of CYP3A1 and CYP3A2 in rats using the western blotting technique. Specific bands were detected at around 50 kDa for CYP3A1 and CYP3A2, which were consistent with the previous reports (Shibayama et al., 2006). Although there is 89% similarity in the sequence of CYP3A1 and CYP3A2 at the amino acid level (Debri et al., 1995), the used antibodies can recognize their own antigens specifically. Results of western blots were in agreement with the results in vitro and in vivo. Pharmacokinetic profiles were similar in the middle- and high-dose triacontanol groups. On the one hand, this observation can reasonably be interpreted by the obvious enhancement of CYP3A2 protein expression level in high-dose group; on the other hand, the expression level of CYP3A1 was lower, compared with that of in the middle-dose group. Consequently, the total protein expression levels of CYP3A1 and CYP3A2 in these two groups were more likely to be similar. Acting as an entirety, CYP3A1 and CYP3A2 participated in the metabolism of docetaxel. Therefore, we conclude that the observed effect of triacontanol on the pharmacokinetics of docetaxel in rats is mainly attributed to the up-regulation of CYP3A1/2 protein.

The impact of triacontanol on CYP3A1/2 mRNA expression was also investigated in rats using the RT-PCR technique. The results of the research showed that triacontanol pretreatment could enhance the mRNA expression level of hepatic CYP3A1, but had little effect on CYP3A2. These results were in accordance with previous report in which dexamethasone, the ligand of pregnane X receptor (PXR), increased CYP3A1 mRNA expression, but insignificantly changed CYP3A2 mRNA (Mei et al., 2004). The absolute mRNA expression level of CYP3A2 in females was lower than that of in male rats (data not shown), because the expression of CYP3A2 mRNA markedly declines with the age of the animals, especially in females (Telhada et al., 1992). However, as far as the pharmacokinetic behaviors of docetaxel was concerned, there were no differences between male and female rats. Furthermore, a certain degree of inconsistency existed between CYP3A1/2 mRNA levels and the protein levels. This discrepancy may be explained that post-transcriptional regulation is slightly different between CYP3A1 and CYP3A2. Moreover, there may be disconnection between the protein and mRNA levels of CYP3A1/2. Therefore, we may reach the conclusion that the induction of CYP3A1 and CYP3A2 is reflected by the up-regulated protein level rather than by the mRNA level (Yu et al., 2008; Zhang et al., 2006).

Nevertheless, it remains unknown what kinds of transcriptional factors mediate the induction of CYP3A1/2 protein expression by triacontanol. Transcriptional factors, such as constitutive androstane receptor and PXR, CCAAT/enhancer-binding proteins, C/EBPα and C/EBPβ, and hepatocyte nuclear factors, as well as HNF4α and HNF3γ, mainly regulated the CYP3A expression at the transcriptional level (Martínez-Jiménez et al., 2007). Therefore, an in vitro human-derived cell-based study is needed to explain CYP3A transcription induced by triacontanol. Further studies are of great importance to better understand the underlying mechanism of transcriptional regulation.

Conclusion

Our study first developed a rat model of long-term administration with triacontanol to assess potential DDI between triacontanol and docetaxel, and to explore the underlying mechanisms of this interaction. In this study, the concentrations of docetaxel in rats were remarkably reduced after administration of triacontanol for seven days, the impact of triacontanol on the pharmacokinetics of docetaxel was largely due to the induction of CYP3A1/2 in the liver. Although species difference may exist, the effects on pharmacokinetics of docetaxel demonstrated that the efficacy of docetaxel will diminish during concomitant administration of triacontanol and docetaxel, this is more likely to lead to the development of cancer resistance in patients. Therefore, the attention should be paid when the combination is administered.

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Declaration of interest

The authors report no declaration of interest.

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